

Inspired by the natural example of the K-ATP channel in which an ion channel (Kir6.2) is regulated by an associated, unrelated membrane protein, the ABC protein SUR, we have engineered Ion-Channel Coupled Receptors (ICCRs) by physical coupling of G-protein coupled receptors (GPCRs) with Kir6.2.

A first ICCR was constructed using the muscarinic M2 receptor and Kir6.2. Our strategy consisted of creating various fusion proteins by linking the receptor C-terminus to the channel N-terminus and progressively removing residues from either termini until functional coupling was achieved, i.e., agonist binding to the receptor modified channel activity. The fusion proteins were heterologously expressed in *Xenopus* oocytes and characterized with the two-electrode voltage-clamp and patch-clamp techniques.

Successful coupling was achieved with limited deletions of the channel N-terminal with an optimum of 25 residues. The optimal construct was reversibly upregulated by the M2 agonist, acetylcholine. To further establish proof-of-concept, a second ICCR was obtained by using the dopaminergic D2 receptor. This ICCR was also regulated by D2 agonists and antagonists although, unexpectedly, the D2 ICCR responses were the opposite of those of the M2 ICCR, i.e., agonists caused channel downregulation.

We observed that, i) agonist modulation of Kir6.2 was concentration-dependent and saturable, ii) agonist effects were abolished by receptor antagonists, iii) the GPCRs within the fusion remained functional as verified by their capacity to activate coexpressed G-protein-activated Kir3 channels. iv) receptor-mediated responses were independent of G-protein activation because they persisted in the presence of pertussis toxin, and v) ICCRs remained functional in cell-free, outside-out patch conditions.

ICCRs could be useful tools for the study of GPCR activation and K⁺ channel gating and could also serve as biosensors for drug screening and diagnostics. Ref :

Moreau et al, Nature Nanotechnology. 2008, in press.

2409-Pos Board B379

KirBac1.1: It's An Inward Rectifying Potassium Channel

Wayland W.L. Cheng¹, Decha Enkvetchakul², Colin G. Nichols¹.

¹Washington University School of Medicine, Saint Louis, MO, USA, ²Saint Louis University School of Medicine, Saint Louis, MO, USA.

The structures of KirBac1.1 and KirBac3.1 have been used extensively to generate in silico homology models of eukaryotic Kir channels and to explore ion permeation, gating and drug-channel interactions by computational approaches. Functional studies of KirBac1.1 have been limited to ⁸⁶Rb⁺ flux assays, but we have now succeeded in measuring voltage-clamp currents of KirBac1.1 reconstituted in giant liposomes. Using the patch-clamp technique, we recapitulate the results of ⁸⁶Rb⁺ flux experiments, showing that KirBac1.1 currents are potassium-selective, blocked by barium, and inhibited by PIP₂. These findings suggest that KirBac1.1 channels are functionally similar to eukaryotic Kir channels. Like the weak inward rectifiers Kir1.1 and Kir6.2, introduction of a negative charge at the "rectification controller" residue in the inner cavity of KirBac1.1 (I138D) confers strong inward rectification (i.e. steep voltage-dependent block by spermine). Steady state single channel currents of KirBac1.1 show multiple subconductance levels and gating modes in 150 mM symmetrical K⁺. However, similar to eukaryotic Kir channels, single channel amplitudes exhibit mild intrinsic inward rectification, with a maximum conductance at ~56 pS (-100 mV), and open probability is higher at positive potentials. Multiple conductance states are still present in single channel currents of other permeant ions such as Rb⁺ and Tl⁺. However, similar to many K⁺ channels, including KcsA, Rb⁺ and Tl⁺ single channel currents show increased mean open time and decreased conductance. We find that KirBac1.1 (T142C), equivalent to the Kir6.2 high P(o) mutant L164C, also has a high open probability and is effectively blocked by Cd²⁺. These electrophysiological results confirm that KirBac1.1 is a bona fide inward rectifying K⁺ channel and a tractable model for study of the molecular basis of inward rectification, permeation and gating in eukaryotic Kir channels.

2410-Pos Board B380

Stabilization of KirBac1.1 Tetramer by Blocking Ions

Shizhen Wang^{1,2}, Yewande Alimi¹, Ailing Tong², Colin G. Nichols², Decha Enkvetchakul¹.

¹Saint Louis University, Saint Louis, MO, USA, ²Washington University, Saint Louis, MO, USA.

Potassium channels are tetrameric proteins that mediate K⁺ selective transmembrane diffusion. For the prototypic potassium channel KcsA, interactions between ions and the channel pore can modulate the stability of the tetrameric structure of KcsA, where permeant and strongly blocking ions increase stability, and impermeant or weakly blocking ions tend to decrease stability. Because

of the structural similarity between the transmembrane regions of KirBac1.1 and KcsA, we examined the role of blocking ions on KirBac1.1 tetramer stability. In 150 mM KCl, purified KirBac1.1 protein migrates as a monomer (~40kD) on SDS-PAGE. Addition of Ba²⁺ (K_{1/2} ~ 50 μM) prior to loading results in an additional tetrameric band (~160 kD). Mutation A109C, at a residue located near the expected Ba²⁺ binding site, decreased tetramer stabilization by Ba²⁺ (K_{1/2} ~ 300 μM), while I131C, located nearby, stabilized tetramers in the absence of Ba²⁺. Neither mutation affected Ba²⁺ block of channel activity (using ⁸⁶Rb⁺ flux assay). In contrast to Ba²⁺, Mg²⁺ had no effect on tetramer stability (even though Mg²⁺ was a potent blocker). Many studies have shown Cd²⁺ block of K channels as a result of introduced cysteines in the cavity-lining M2 (S6) residues, with the implicit interpretation that coordination of a single ion by cysteine side-chains along the central axis effectively blocks the pore. We examined blocking and tetramer stabilizing effect of Cd²⁺ on KirBac1.1 with cysteine substitutions in M2. Cd²⁺ block potency followed an alpha-helical pattern consistent with the crystal structure. Significantly, Cd²⁺ strongly stabilized tetramers of I138C, located in the center of the inner cavity. This stabilization was additive with the effect of Ba²⁺, consistent with both ions simultaneously occupying the channel; Ba²⁺ at the selectivity filter entrance and Cd²⁺ coordinated by I138C side-chains in the inner cavity.

2411-Pos Board B381

Kirbac 1.1 activity in liposomes is suppressed by cholesterol

Dev K. Singh¹, Decha Enkvetchakul², Colin G. Nichols², Irena Levitan¹.

¹University of ILLINOIS, Chicago, IL, USA, ²Washington University, Saint Louis, MO, USA.

Inwardly rectifying potassium channels (Kir) are responsible for regulating diverse processes including: cellular excitability, vascular tone, heart rate, renal salt flow, and insulin release. Our earlier studies have shown that inwardly-rectifying K channels from Kir2 family are strongly suppressed by the elevation of cellular cholesterol. Little is known, however, about the mechanism responsible for cholesterol modulation of Kir channels. The goal of this study is to test whether cholesterol-induced suppression of Kir channels is observed when purified channels are incorporated into liposomes. To achieve this goal, we reconstituted a bacterial channel Kirbac 1.1, a structural homolog of mammalian Kir channels, into liposomes of defined composition consisting of 3:1 phosphatidyl ethanolamine: phosphatidyl glycerol ratio and variable concentrations of cholesterol. The activity of the channels was assayed using ⁸⁶Rb⁺ uptake. Our results show that ⁸⁶Rb⁺ flux through the Kirbac 1.1 is strongly inhibited by incorporating cholesterol. Incorporation of 5% (mass Cho/PL) cholesterol into the liposome suppresses more than 50% ⁸⁶Rb⁺ flux, and the activity is completely inhibited at 12-15% (mass Cho/PL). No effect was observed at cholesterol levels below 1% (mass Cho/PL). Furthermore, epicholesterol, a stereo isomer of cholesterol that has physical properties similar to those of cholesterol, also suppresses ⁸⁶Rb⁺ flux but its effect is significantly less pronounced. Purified KcsA, structurally similar K⁺ ion channel from *Streptomyces lividans* was not at all inhibited by cholesterol when incorporated into the liposomes instead of Kirbac 1.1. These observations demonstrate that cholesterol suppresses Kir channels in a non-cellular environment and suggest that it may interact with the Kirbac 1.1 channels directly.

2412-Pos Board B382

Physical Determinants of Strong Voltage Dependence of K⁺ Channel Block

Yanping Xu¹, Hyeon-Gyu Shin¹, Zhe Lu².

¹University of Pennsylvania, Philadelphia, PA, USA, ²Howard Hughes Medical Institute; University of Pennsylvania, Philadelphia, PA, USA.

Many pharmacological agents, as well as some endogenous biological molecules, act by blocking ion channels in a strongly voltage-dependent manner. In retrospect, the first and most dramatic example of such action is the "anomalous" voltage dependence of inwardly rectifying K⁺ (Kir) conductance discovered by Bernard Katz six decades ago. He observed that, contrary to the classic voltage-gated K⁺ conductance, the Kir conductance tends to zero with membrane depolarization but increases with hyperpolarization. In intact cells and within physiological voltage ranges, certain Kir channels are as steeply voltage dependent as Kv channels yet, unlike Kv channels, they have no inherent voltage sensors: the observed voltage sensitivity instead reflects voltage-dependent block of their ion pore by intracellular cations such as the polyamine spermine. Our group has proposed that the high valence associated with block of strong rectifiers primarily reflects the movement, not of tetravalent spermine itself, but of five K⁺ ions displaced by spermine across the steep electric field in the narrow K⁺ selectivity filter. We will present experimental evidence for key requirements of this blocker-K⁺ displacement model, and will discuss the essential features that render a pore-blocking process strongly voltage dependent.